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SNR1 is an essential subunit in a subset of *Drosophila* brm complexes, targeting specific functions during development

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Abstract

The *snr1* gene of *Drosophila melanogaster* encodes a conserved component of the multiprotein Brahma (Brm) complex, a counterpart to the SWI/SNF complexes that participate in ATP-dependent chromatin remodeling. Loss-of-function and null mutations in the *snr1* gene reveal its essential role in *Drosophila* development. We identified new mutant alleles and ectopically expressed deleted forms to dissect the specific functions of SNR1. Somatic and germ cell clone analyses confirmed its requirement in a continuous and widespread fashion for proper cell fate determination and oogenesis. Expression of SNR1 transgenes revealed unexpected roles in wing patterning, abdomen development, oogenesis, and sustained adult viability. A widespread distribution of SNR1 and BRM on the salivary gland polytene chromosomes showed that the Brm complex associated with many genes, but not always at transcribed loci, consistent with genetic data suggesting roles in both gene activation and repression. Despite essential Brm complex functions in leg development, genetic and protein localization studies revealed that *snr1* was not required or expressed in all tissues dependent on Brm complex activities. Thus, SNR1 is essential for some, but not all Brm functions, and it likely serves as an optional subunit, directing Brm complex activity to specific gene loci or cellular processes.

Keywords: *Drosophila*; SWI/SNF; Chromatin; Brahma; SNR1; INI1; SNF5; PolII; BRM; Polytene

Introduction

Throughout metazoan development, groups of genes are activated and repressed in distinct temporally and spatially restricted patterns. Cell determination requires that specific genes be targeted for induced expression, or silenced, in response to growth signals. The competence of a gene to be expressed is determined by inductive events during development, and once established, this competence is epigenetically heritable through subsequent cell divisions, in some instances maintained through the maternal germline (Cavalli and Paro, 1998, 1999). This type of cellular memory is somehow "imprinted" through affects on chromatin architecture. In all eukaryotic cells, chromatin structure serves to control the accessibility of transcription regulatory proteins to specific sites and imposes a default state in which genetic information is repressed by higher order structures. The SWI/SNF complexes, identified in yeast, flies (Brm complex), and mammals (hBrm and BRG1 complexes), are large (~2 MDa) multisubunit assemblies (8–11 proteins) that alter or remodel the structure of chromatin in an energy-dependent fashion.

While the biochemical properties of the purified yeast and human SWI/SNF complexes have been studied in great detail (Kingston et al., 1996; Kingston and Narlikar, 1999), the in vivo functions are less well understood. The SWI2/SNF2 ATPase subunit alone is partially sufficient for nucleosome disruption in vitro, and only 4 subunits of the mammalian complex, BRG1, INI1/hSNF5, BAF155, and

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BAF170, are required for full in vitro ATP-dependent chromatin remodeling activity (Phelan et al., 1999). Therefore, the additional 5–11 subunits may modulate complex functions or provide target specificity. The SWI/SNF complex can be recruited to specific sites through direct interactions with gene-specific activators (Peterson and Workman, 2000) with several complex subunits functioning coordinately to facilitate these associations (Neely et al., 1999, 2002); however, little is known regarding the specific protein interactions that promote such recruitment.

Components of the *Drosophila* Brm complex were initially identified through genetic screens for regulators of homeotic (HOM) gene expression (Tamkun, 1995; Kennison and Tamkun, 1988) affecting leg development. The *brm*, *mor*, and *osa* genes serve as activators to counteract the effects of the Polycomb group (Pc-G) of genes that maintain the repressed state of HOM gene expression. Homology between BRM and yeast SWI2/SNF2 within the ATPase domain and the discovery of *snr1*, a fly homolog of *SNF5*, strongly suggested that a counterpart to the SWI/SNF complex was involved in maintaining active HOM gene expression in flies (Dingwall et al., 1995; Tamkun et al., 1992). The Brm complex purified from embryos is comprised of 8–10 stably associated subunits, including BRM, MOR, SNR1, and OSA (Collins et al., 1999; Crosby et al., 1999; Dingwall et al., 1995; Papoulas et al., 1998). While *brm*, *mor*, and *osa* are dosage-limiting for most Brm complex functions, no other subunit was identified in any genetic screen. One hypothesis is that the additional Brm complex subunits, including *snr1*, are important for restricted or limited functions, perhaps regulating locus- or process-specific complex activities, and thus might not be continuously required in stoichiometric quantities in all tissues. An attractive alternative hypothesis is that other than a "catalytic core," which includes the BRM ATPase, some components may be optional and thus either transiently associated or not present in all tissues where Brm complex functions have been identified.

The *snr1* gene (*SNF5-Related-1*) is highly conserved with counterparts in both mammals (*INI1/SMARCB1/hSNF5*) and yeast (*SNF5*). While mutations in *SNF5* are not lethal, the *snr1* and *INI1* genes are essential for normal development (Dingwall et al., 1995; Klochendler-Yeivin et al., 2000). Further, biallelic disruption of *INI1*, but no other mammalian hBrm or Brg1 complex gene, is strongly (~90%) associated with aggressive rhabdoid (MRT) tumors (Sevenet et al., 1999; Versteege et al., 1998), suggesting that *INI1* serves as a tumor suppressor. Although several studies have suggested that SNR1 and INI1 are stoichiometric core components in their respective SWI/SNF-type complexes (Papoulas et al., 1998; Phelan et al., 1999; Wang et al., 1996), there is mounting genetic evidence suggesting that *snr1* and *INI1* may not be required for all complex functions, challenging definitions of a "core" subunit. For example, although mutations in *snr1* exhibit genetic interactions with *brm*, *osa*, and *trx* in the dorsal prothorax, eyes, and abdomen (Collins et al., 1999; Dingwall et al., 1995), it does not appear to be dosage-limiting for Brm complex functions in the leg (Bajusz et al., 2001; Dingwall et al., 1995; Kennison and Tamkun, 1988), a test that is often used to define members of the trx-G of gene activators (Kennison, 1995). Thus, the lack of *snr1* mutant dosage-sensitivity was surprising, suggesting that SNR (INI1) is only required for a limited set of Brm complex functions or that it has activities independent of the complex. To distinguish among these and other possibilities, we employed genetic screens to identify new *snr1* mutant alleles, examined somatic and germline null mutant clones, and ectopically expressed SNR1 deletions. Our results demonstrate that *snr1* is essential and its function is important for oogenesis and proper development of a restricted set of tissues, including the peripheral nervous system, abdomen, and wings and an unanticipated role in sustaining adult viability; however, *snr1* is not required for proper leg development. In support of our genetic studies, *snr1* mRNA is present in adult males, and while SNR1 is broadly expressed, it is not detectably present in a subset of tissues where BRM is found, including the leg discs (Elfring et al., 1998). These genetic and expression data are consistent with the hypothesis that SNR1 is an optional component required for Brm complex functions in restricted tissues; in particular, by several criteria, SNR1 does not appear to be important for critical Brm complex functions in regulating genes involved in leg development. Moreover, we show that SNR1 and BRM are present together at many locations on the salivary gland polytene chromosomes often overlapping with RNA Polymerase II, suggesting a wide array of potential in vivo targets for gene regulation. The Brm complex and PolII are also found at a limited number of nonoverlapping chromosomal sites, raising the possibility that the Brm complex might be involved in regulating other cellular processes through effects on chromatin structure.

Materials and methods

Fly strains and media

Flies were raised on standard cornmeal/dextrose medium at 25°C unless otherwise noted. The mutations and chromosome aberrations used are shown in Fig. 1 or described in FlyBase (http://flybase.bio.indiana.edu). Mutant alleles of *snr1* were induced by ethane methylsulfonate (EMS) mutagenesis (Zraly et al., 2002) or *P*-element excision (Dingwall et al., 1995). Accessory recessive mutations were removed by recombination with a multiply marked third chromosome (st^I, Ki^I, p^P) and verified by transgene rescue.

Generation of rescue and snr1 deletion transgene lines

A genomic phage library (Tamkun et al., 1992) was screened by using a *snr1* cDNA. An 8.9-kilobase (kb)

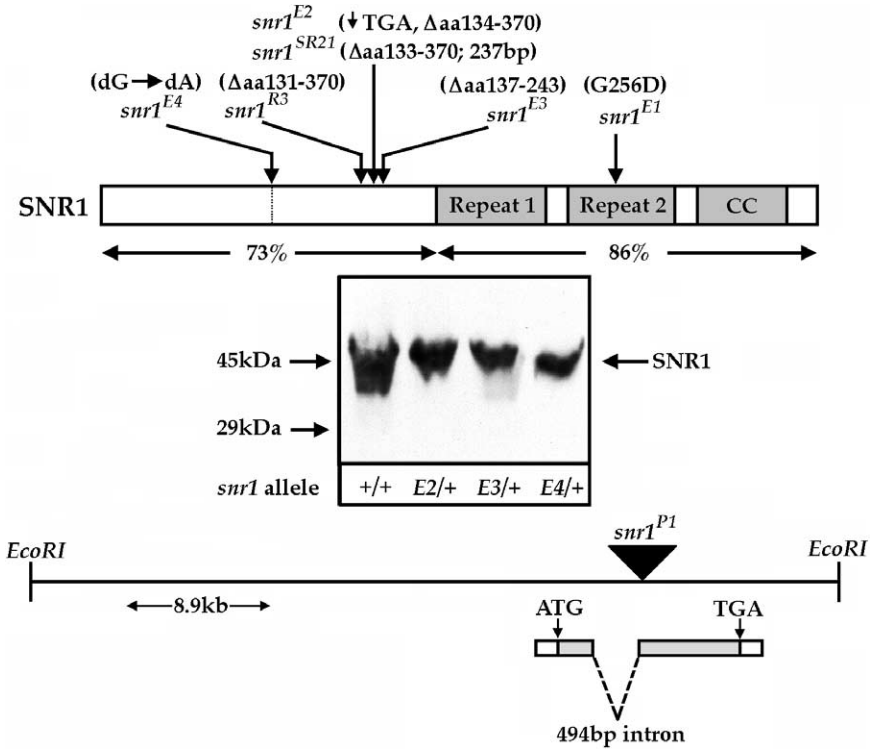

Fig. 1. Molecular analysis of *snr1* mutations. (Top) SNR1 protein structure, conservation with INI1 (% identity), and location of mutations. Conserved regions among SNF5-family members include two Repeat domains that mediate critical protein interactions and a coiled coil. (Middle) *snr1* mutations are nulls by Western blot. Reduced protein levels and no truncated forms are observed in extracts prepared from *snr1* mutant strains. (Bottom) Genomic region used for rescue transgene constructs and location of *snr1*.

*Eco*RI restriction fragment containing 5.7 kb of genomic DNA 5′ and 1 kb 3′ to *snr1* was cloned into a *P* transformation vector (Pirrotta, 1988). Transgenic lines *(w, P[w$^+$, gr.snr1$^+$])* were established and insertions mapped to specific chromosomes. An inducible full-length *snr1* cDNA was generated by using a 1.4-kb *Ssp*I–*Not*I fragment cloned into pCaSpeR-hs and transgene lines established. A second (II) chromosome transgene was used for *snr1* cDNA rescue analyses *(w; P[w$^+$, hs.snr1])*, with expression verified by Western blot analysis of embryo extracts following heat shock (data not shown).

Molecular analysis of new snr1 alleles

Genomic DNA was prepared from *snr1* mutant first instar larvae (Garazzo and Christenson, 1994; Gloor and Engels, 1992). The *snr1* gene was amplified with *ExTaq* polymerase (Takara) by using gene-specific primers, and products were gel-purified and fully sequenced (BioResource Center, Cornell University).

Protein extracts were prepared from control and *snr1* mutant pupae (Dingwall et al., 1995; Zraly et al., 2002), fractionated by 10% SDS–PAGE, then probed with anti-SNR1 antibodies. Goat anti-rabbit HRP-conjugated secondary antibody (Jackson ImmunoResearch) and enhanced chemiluminescence (Pierce) were used for detection.

Mosaic analyses

The FLP/FRT system was used to induce somatic and germline clones of a strong loss-of-function *snr1^{R3}* mutation, as described (Golic and Lindquist, 1989; Xu and Rubin, 1993). To generate a recombinant chromosome, *w^{1118}; P[ry$^+$, hs-neo, FRT]82B* flies were crossed with *w^{1118}; P[w$^+$]82F*. G418-resistant *(neo^r)* recombinants *(w^{1118}; P[ry$^+$, hs-neo, FRT]82B, P[w$^+$]82F)* were selected and crossed to *w^{1118}; snr1^{R3}/TM6B*. Recombinants *(w^{1118}; P[ry$^+$, hs-neo, FRT]82B, snr1^{R3})* were recovered at low frequency ($\sim 1 \times 10^{-4}$) and verified by PCR using *neo*-specific primers, complementation tests, and transgene rescue.

Somatic clones were generated by using *hs-FLP* recombinase carried on the first chromosome *(y, w, P[hs-FLP])*. Clones were induced in flies of the genotype *(y, w, P[hs-FLP]; P[ry$^+$, hs-neo, FRT]82B, snr1^{R3}/P[ry$^+$, hs-neo, FRT]82B, P[ry$^+$, y$^+$]96E, Sb)* by exposing larvae to a 37°C heat-shock for 1 h, followed by recovery and incubation at 25°C until eclosion. Clones (*snr1* −/−) were scored based on the appearance of *y*, non-*Sb* bristles. As controls, clones were produced in parallel from chromosomes that carried the FRT alone and from non-heat-shock animals. Abnormal bristle phenotypes were scored as the appearance of shortened, bent, fused, twinned, or missing bristles based on the

criteria used previously to score *brm* clones (Elfring et al., 1998).

The production of germline clones followed standard procedures (Chou and Perrimon, 1996). Larvae were heat-shocked at 37°C for 60 min during both the first and third instar larval periods. Virgin females of the appropriate genotype *(y, w, P[hs-FLP]; P[ry$^+$, hs-neo, FRT]82B, snr1^{R3}/ P[ry$^+$, hs-neo, FRT]82B, P[w^{+mC}, ovo^{D1-18}]C13X3a)* were crossed to *w* males and allowed to lay eggs for 14 days. Control crosses were tested in parallel by using appropriately marked females *(y, w, P[hs-FLP]; P[ry$^+$, hs-neo, FRT]82B, P[y$^+$], Sb/P[ry$^+$, hs-neo, FRT]82B, P[w^{+mC}, ovo^{D1-18}]C13X3a)*.

Ectopic expression of snr1 transgenes and rescue analyses

Females *(w^{1118}, P[w$^+$, gr.snr1];TM3/TM6B or w^{1118}; P[w$^+$, gr.snr1];TM3/TM6B)* were crossed to *snr1* mutant males *(w^{1118}, snr1*/TM6B)*. Male progeny carrying both the transgene and the *snr1* allele were then crossed to either the same or another mutant allele to assess rescue frequency. The full-length *snr1* cDNA under the control of the *HSP70* heat-shock promoter was induced at 37°C for 30 min every 12 h during development *(w^{1118};P[w$^+$, hsp70-snr1];snr1*/ TM6B)*. Rescue ability was measured by comparing with non-heat-shocked flies of the same genotypes.

Deletions of the *snr1* cDNA were constructed and cloned into pUAST (Brand et al., 1994). *UAS:snr1-1* (aa 1–370) contains a *Ssp*I–*Not*I fragment that includes the full-length *snr1*cDNA (including both 5′ and 3′ UTRs), *UAS:snr1-2* (aa 1–261; also known as *snr1-cdel.3*; Brumby et al., 2002), *UAS:snr1-3* (aa 1–155), and *UAS:snr1-4* (aa 1–44). All constructs retain the *snr1* 5′ UTR (see Fig. 3). At least two independent transgene lines were generated for each construct. Ectopic expression was carried out by using the GAL4/UAS binary system (Brand et al., 1994; van Roessel and Brand, 2000). Flies bearing GAL4 driver transgenes that produce GAL4 protein either ubiquitously (*Actin5C* or *HSP70*) or in restricted fashion were crossed to responder flies carrying the *GAL4-UAS:snr1* transgenes. At least 30 different GAL4 driver lines were tested in this fashion with 2 independent *snr1* responder lines carrying the same transgene construct. The effects of *snr1* gene dosage on expression phenotypes were assessed in flies bearing the responder transgenes together with a *snr1* null allele *(snr1^{R3})*. All crosses were performed at 29°C unless otherwise indicated. Expression of each transgene was determined by Western blot using extracts from embryos (0–24 h after egg laying) or pupae (24–48 h after pupariation) as described previously (Zraly et al., 2002). Whole cell, cytoplasmic, and nuclear extracts were prepared (Moritz, 2000; Pazin, 2000) from pupae ubiquitously expressing SNR-1, SNR1-2, or control pupae raised at 29°C. Proteins were fractionated by SDS–PAGE, blotted, and probed with anti-SNR1 and anti-tubulin (Babco) antibodies. Immunoprecipitations of SNR1, SNR1-2, and BRM were performed by using embryonic extracts as described (Dingwall et al., 1995).

Production of antibodies and immunostaining

Affinity purified rabbit polyclonal SNR1 antiserum was produced by immunizing rabbits with a full-length (aa 2–370) SNR1:HIS fusion (produced in pTrcHis; Invitrogen). SNR1-specific antibodies were affinity purified by using the SNR1$^{2–370}$:HIS fusion bound to AminoLink resin (Pierce Endogen, Inc). Controls for SNR1 antibody specificity included testing different portions of SNR1 expressed in bacteria as fusion proteins, with all regions capable of being recognized (data not shown). Also, third instar larvae carrying a heat-inducible *snr1* cDNA rescue transgene *(hsp70-snr1)* were heat-shocked for 60 min followed by a 30-min recovery. Both heat-shocked and control larvae were dissected in 1 × PBS, 0.1% Triton X-100 (PBST) and fixed, and imaginal discs were immunostained with the SNR1 antibody. BRM-specific antibodies (4449.3) were raised in rabbits immunized with an amino-terminal fragment of the BRM protein fused to GST (GST:BRM$^{3–100}$) and affinity purified.

Third instar Oregon R larvae raised at 25°C were dissected in PBST and fixed, and tissues were immunostained with anti-SNR1 or anti-BRM antibodies diluted in PBSTB (1× PBS, 0.1% Triton X-100, 0.2% BSA). Ovaries from 4 days post-eclosion wild type females held on fresh yeast were dissected in Ringer's, fixed (Matthies et al., 2000), and immunostained with anti-SNR1 antibody. Donkey anti-rabbit HRP secondary antibodies (Jackson ImmunoResearch) were used for detection. Immunolocalization of SNR1, BRM, and RNA PolII on third larval instar salivary gland polytene chromosomes was performed with affinity-purified primary antibodies (α-SNR1, 1:100; α-BRM, 1:250 diluted in blocking solution). The goat α-RNA PolII gAP alpha-D1 antibody (gift of A. Greenleaf) recognizes RpII140, the second largest subunit of *Drosophila* PolII (IIc) as described (Skantar and Greenleaf, 1995) and was used at a dilution of 1:100 in blocking solution. Polytene chromosome immunostaining was performed as described (Zink and Paro, 1989, 1995) with detection carried out using Cy-3-conjugated donkey anti-rabbit (1:350) or Cy-2-conjugated donkey anti-goat (1:100) secondary antibodies (Jackson ImmunoResearch) diluted in block solution supplemented with 2% normal donkey serum.

PEV analysis

Females carrying a variegating allele of the *white* gene *(w^{m4h})* were crossed to males carrying mutations in Brm complex genes or controls and incubated at 25°C. Newly eclosed nonbalanced male progeny were selected and held for 3 days at 25°C, then stored at −80°C. Heads from 20 males per sample (with at least 2 replicate samples) were homogenized in 50 μl of acidified ethanol (30%, pH 2.0),

then the volume was raised to a total of 1500 μl. Extractions were incubated in the dark at 25°C for 48 h, then pigments were quantified by absorbance at 480 nm. *snr1* mutants used for this assay were derived from *P*-excisions and included nonsense *($snr1^{R3}$)*, internal deletion *($snr1^{SR21}$)*, deficiency *($snr1^{SR71}$)*, and viable precise excision *($snr1^{PIrev}$)* alleles (Dingwall et al., 1995).

Results

Characterization of snr1 mutant alleles

Existing mutant alleles of *snr1* include a *P*-element insertion *($snr1^{P1}$)* and a *P*-excision truncation *($snr1^{R3}$)*. Both disrupt SNR1 at aa 131, eliminating most of the highly conserved regions, and result in recessive lethality during the late first or early second larval instar stage (Dingwall et al., 1995; Zraly et al., 2002). An 8.9-kb *snr1* genomic fragment was isolated and cloned into pCaSpeR-hs to generate rescue transgenes located on either the first or second chromosomes. Transgenes on either chromosome could rescue the recessive lethality (data not shown). Heat shock expression of a full-length *snr1* cDNA was also capable of rescuing lethality; however, rescued flies exhibited significantly shortened life spans (<5 days post-eclosion). Although these alleles are likely nulls, we repeated the *P*-mobilization screen to generate small deficiencies. Among nearly 150 excision lines, we identified a small internal deletion of *snr1 ($snr1^{SR21}$)* and two small deficiencies surrounding the *snr1* gene *($snr1^{SR29}$, $snr1^{SR71}$)*. The $snr1^{SR21}$ lethality was rescued by a genomic transgene while the deficiency lines were not, indicating that the deletions removed other vital genes. Sequencing of the $snr1^{SR21}$ mutation revealed a 237-bp deletion and frame shift, truncating SNR1 at Thr133. In genetic tests, $snr1^{SR21}$ behaved as a null (data not shown). Southern blots of $snr1^{SR29}$ and $snr1^{SR71}$ strains revealed deletion of *snr1* and portions of the surrounding genomic DNA.

A noncomplementation (EMS) screen identified four new recessive lethal alleles of *snr1* (Zraly et al., 2002) that failed to complement both $snr1^{R3}$ and $snr1^{SR21}$. All were rescued as heterozygotes in trans with $snr1^{R3}$ and as homozygotes. Homozygous and trans-heterozygous mutant combinations exhibited lethality at the end of the first (L1) or early second (L2) larval instar stage with no obvious developmental defects. Sequencing (Fig. 1) revealed that *E2* resulted from a 31-bp insertion; including a direct duplication of 10 bp, likely caused by insertion then imprecise excision of a mobile element. The inserted DNA produces an in-frame stop (TGA) at aa 134, close to the position of our lethal *P*-insertion, suggesting a transposition hotspot. The *E3* mutation deletes the region encoding aa 137 to aa 243, including Repeat 1, although the open reading frame remains intact; *E4* is a single base change (G/A) at the *snr1* intron 5′ splice donor site. While a cryptic splice site might be used, this seems unlikely as there is no other consensus 5′ donor site and failure to splice properly would lead immediately to a TAA stop codon following aa 91. Characterization of the $snr1^{E1}$ allele is reported elsewhere (Marenda et al., 2003).

The molecular weights of the predicted $SNR1^{R3}$ (14.4 kDa), $SNR1^{E2}$ (14.6 kDa), $SNR1^{E3}$ (29 kDa), and $SNR1^{E4}$ (10 kDa) proteins are significantly smaller than the native SNR1. Extracts from heterozygous $snr1^{E2}$, $snr1^{E3}$, and $snr1^{E4}$ mutant pupae were tested by Western blot. Polyclonal antibodies to SNR1 recognize a prominent 45-kDa species representing wild-type protein (Fig. 1) and are capable of recognizing the predicted mutant proteins (data not shown). None of the *snr1* alleles tested produced any detectable protein (Fig. 1, and data not shown), indicating that all are null mutations.

Maternal and zygotic SNR1 functions are essential

To examine tissue-specific *snr1* loss-of-function phenotypes, recombinant chromosomes were generated to produce somatic and germline clones by using the FLP/FRT system (Chou and Perrimon, 1996; Golic, 1991; Xu and Rubin, 1993). Somatic cell-derived *snr1* mutant clones were generated in both a brm^{+} and a brm^{2} heterozygous mutant background. The size and frequency of the *snr1* clones were compared with controls, either non-heat-shocked siblings or clones generated from *FRT*-only chromosomes. A total of 1165 *snr1* clones were scored and compared with 375 control clones (Table 1). Mutant clones were more frequently observed at late larval stages and clone sizes were generally larger when induced during L3 compared with L2, possibly indicating that *snr1* mRNA and protein produced during the L1 and L2 stages might persist to allow for continued cell division after clone induction. However, both frequency and size were reduced relative to control clones, revealing that *snr1* was important for cell viability. Mutant clones generated in a heterozygous brm^{2} background were less frequently observed, and clone sizes were reduced compared with both brm^{+} and control clones induced at similar stages, suggesting an additive effect between *brm* and *snr1*.

Examination of *snr1* clones in the abdomen revealed a strong similarity with previously described *brm* clones (Elfring et al., 1998), including duplicated, fused, or malformed (stunted, twisted or bent) mechanosensory bristles (Fig. 2). Within *snr1* clone boundaries, but not controls, patches of tissue were observed that were missing bristles and/or bristle sockets; however, no homeotic transformations were observed. Consistent with *brm* mutant clones, the loss of *snr1* appears to affect peripheral nervous system development. Unlike *brm* clones, *snr1* clones exhibited cuticle defects within the abdominal segments, including fusion of adjacent segments within clones, loss of pigmentation, and cuticle disruptions (~65% of clones scored). The presence of a heterozygous brm^{2} null allele enhanced the *snr1* clone phenotype in the abdomen, suggesting a role for

Table 1
Somatic clonal analysis of *snr1*^{R3}

Structure	No. clones/structures scored				Frequency				Size			Percent of abnormal bristles[a]		
	Non-HS	L1	L2	L3	Non-HS	L1	L2	L3	L1	L2	L3	L1	L2	L3
A. *FRT[82B], snr1*^{R3}														
Head	0/20	2/27	3/18	56/122	0	0.07	0.16	0.45	1.0	1.0	1.0	0	0	100
Dorsal thorax	1/20	9/27	7/18	225/122	0.05	0.33	0.39	1.84	1.0	1.14	2.55	28	0	90
Abdomen	5/20	52/27	83/18	497/122	0.25	1.9	4.6	4.1	1.39	2.7	3.08	85	54	77
B. *brm*^{2} *FRT[82B], snr1*^{R3}														
Head	0/25	3/32	0/21	11/59	0	0.09	0	0.19	1.0	—	1.3	0	—	71
Dorsal thorax	4/25	4/32	0/21	27/59	0.16	0.13	0	0.46	1.5	—	1.85	75	—	100
Abdomen	5/25	20/32	40/21	126/59	0.20	0.63	1.90	2.14	2.5	1.63	2.10	100	100	100
C. *FRT[82B]* control														
Head	1/41	0/20	0/20	10/21	0.02	0	0	0.48	—	—	1.11	0	0	0
Dorsal thorax	3/41	1/20	29/20	82/21	0.07	0.05	1.45	3.90	2.45	7.10	4.20	0	0	0
Abdomen	19/41	2/20	57/20	124/20	0.46	0.10	2.85	5.90	1.50	3.90	4.20	0	0	0

[a] Abnormal bristles within the abdomen were broadly defined as any deviation from wild type, including shortened, twinned, missing, bent or malformed. The primary mutant phenotype observed within clone boundaries on the dorsal thorax was the appearance of slightly shortened bristles relative to wild type.

the Brm complex in histoblast proliferation. Production of *snr1* clones exclusively in the eye imaginal discs (Stowers and Schwarz, 1999) revealed only slightly reduced eye sizes, but no significant morphological defects (data not shown). Few clones were obtained in the head region under any of the conditions tested, and clones on the dorsal thorax only revealed minor shortening of bristles within the clone boundaries, but no other bristle or homeotic phenotypes. Thus, although *brm* is important for the development of the thorax (Elfring et al., 1998), clones of *snr1* did not reveal any clear role. Unlike *brm* clonal analyses, in an examination of ~1600 legs in which *snr1* clones were produced (including a *brm*-sensitized background), we did not observe any obvious phenotypes and clones appeared with a frequency similar to controls (data not shown). As the maternal contribution of *snr1* is sufficient for the completion of development through the early larval stages (Dingwall et al., 1995), it is possible that *snr1* mRNA or protein might persist from expression earlier in development masking potential loss-of-function thoracic phenotypes, and/or that *snr1* may not be critically required for all Brm complex functions.

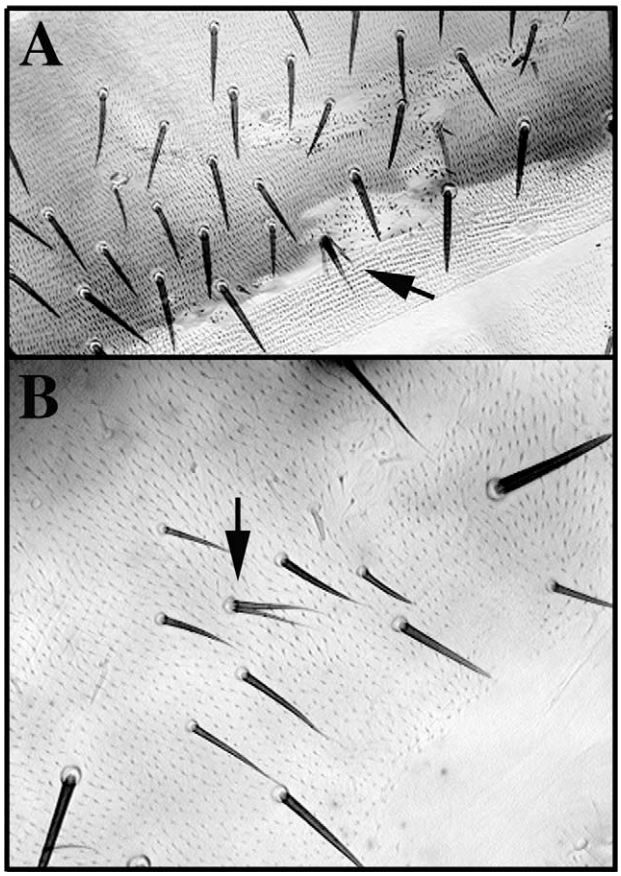

Fig. 2. Mosaic clones of *snr1* reveal bristle-patterning defects. (A) Abdominal cuticle disruptions and malformed bristles (arrow) are observed in *snr1* mosaic clones. (B) Stunted and duplicated bristles in a *snr1* clone on the abdomen.

In contrast to mutant alleles of *brm*, *mor*, and *osa* that revealed critical requirements for the Brm complex in embryogenesis, *snr1* null mutants die as early larvae with no obvious defects. Thus, either the large maternal contribution of *snr1* mRNA is sufficient to allow for normal embryonic development or *snr1* is not required for all Brm complex functions in embryos. To examine possible embryonic roles for *snr1*, female germ cells lacking *snr1* gene product were generated by using the FLP/FRT system combined with the *ovo*^{D1} dominant female sterile mutation that blocks oogenesis (Chou et al., 1993). Heat-shock-induced expression of the *FLP* recombinase during both the first and third larval instar stages allowed for recombination between the *FRT[82B], P[ovo*^{D1}*]* and *FRT[82B], snr1*^{R3} chromosomes producing females with homozygous *snr1* mutant germline cells that had lost the *ovo*^{D1} mutation. A total of 32 females were examined and all were found to be infertile, producing no eggs. Control flies of the genotype *FRT[82B], P[ovo*^{D1}*]/*

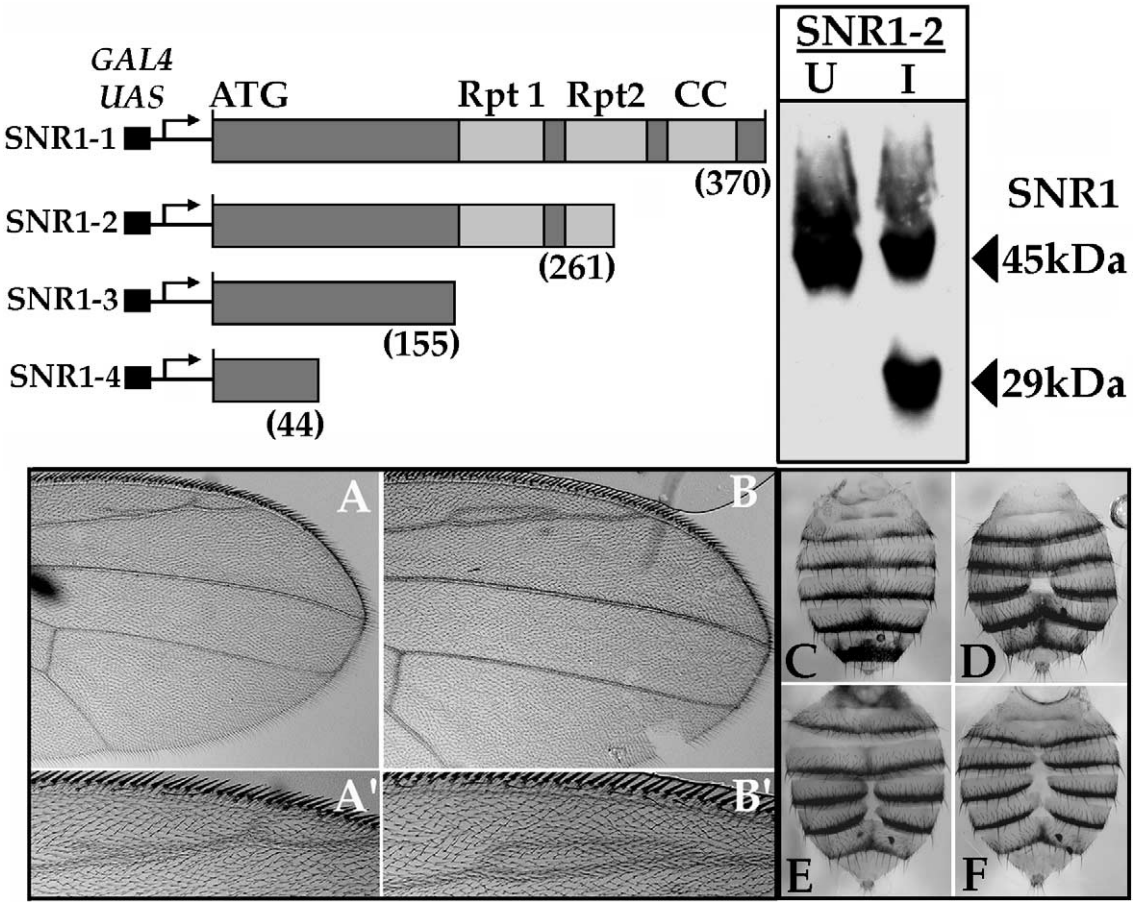

Fig. 3. SNR1 is important for wing and abdomen development. (Left) SNR1 full-length (SNR1-1) or deletion transgene constructs (SNR1-2, -3, -4) were expressed under GAL4 control. (Right) SNR1-2 is under stringent control, induced only by coexpression of GAL4 in transgenic flies. Western blot analysis using anti-SNR1 antibody revealed the 45-kDa SNR1 protein in both uninduced (U) and induced (I) extracts and the 29-kDa SNR1-2 protein only in the induced extracts. SNR1-2 expression phenotypes include L2 wing vein disruptions (A, B) and incomplete dorsal abdomen fusion (D–F). A wild type female abdomen is shown in (C). Severity correlates with temperature: (+) at 18°C (D), (++) at 25°C (E), and (+++) at 29°C (F).

FRT[82B] produced germline mosaic females that were fully fertile (7 out of 7 examined; 198 total eggs produced). Consistent with the heat-shock cDNA rescue results, where all rescued females were infertile once *snr1* product was no longer produced (20 females tested, 0 eggs produced), germline clone analyses revealed that *snr1* is required for oogenesis.

Late developmental functions of snr1

Ectopic expression of *brm* and *osa* has proven useful for examining potential Brm complex functions in larval tissues (Collins et al., 1999; Elfring et al., 1998). To disrupt SNR1 functions in specific tissues, deletions of the *snr1* cDNA removing one or both of the conserved repeat regions (Fig. 3), as well as a full-length cDNA, were placed under the control of a GAL4 responsive enhancer, and independent viable transgene lines were generated. Each construct was tested for expression and examined for phenotypes following GAL4 induction. The SNR1-1 and SNR1-2 proteins were stably expressed at elevated levels relative to wild-type SNR1 based on Western blots probing extracts obtained from *P[w$^+$, snr1Δ]/Act5C-GAL4 (II)* embryos (Fig. 3); however, the SNR1-3 and SNR1-4 proteins were too small or unstable to be detected (data not shown). Although expression of SNR1 is normally restricted to the CNS and brain in late embryos and imaginal tissues in larvae, expression of SNR1-1 with 30 different tissue-specific and ubiquitous GAL4 driver lines had no obvious mutant phenotype, suggesting that SNR1 had no significant function independent of the Brm complex. Expression of large SNR1 deletions (SNR1-3, SNR1-4) with a variety of GAL4 drivers did not produce specific phenotypes, indicating that the truncated proteins did not interfere with normal SNR1 function, consistent with existing *snr1* null alleles that are fully recessive.

In contrast, ectopic expression of SNR1-2 showed significant developmental defects. The GAL4 driver-dependent phenotypes included specific wing vein disruptions (Table 2; Fig. 3C and D), incomplete abdominal tergite fusion along the dorsal midline (Fig. 3F–H), both general and male-specific lethality, and a decrease in adult longevity

Table 2
Abdomen and wing phenotypes resulting from SNR1-2 expression

Temp	Genotype	Total flies	Percentage showing phenotype					
			Abdomen				Wing	
			None	+	++	+++	L2 vein disrupt	L3 bristle
18°C	*UAS:snr1-2a/e22c-GAL4; snr1^{R3}/+*	31	32	26	16	26		
	UAS:snr1-2a/e22c-GAL4; +/TM6B	21	67	24	9	0		
	Control siblings	46	100	0	0	0		
	UAS:snr1-2a; GaWB-69B/snr1^{R3}	82					0	0
	UAS:snr1-2a; GaWB-69B/TM6B	72					0	0
25°C	*UAS:snr1-2a/e22c-GAL4; snr1^{R3}/+*	44	43	18	18	21		
	UAS:snr1-2a/e22c-GAL4; +/TM6B	44	82	11	5	2		
	Control siblings	96	100	0	0	0		
	UAS:snr1-2a; GaWB-69B/snr1^{R3}	94					2	5
	UAS:snr1-2a; GaWB-69B/TM6B	102					0	0
29°C	*UAS:snr1-2a/e22c-GAL4; snr1^{R3}/+*	0	0	0	0	0		
	UAS:snr1-2a/e22c-GAL4; +/TM6B	96	12	32	23	32		
	Control siblings	155	100	0	0	0		
	UAS:snr1-2a; GaWB-69B/snr1^{R3}	65					54	25
	UAS:snr1-2a; GaWB-69B/TM6B	68					0	0
	GaWB-69B/UAS:snr1-2b, snr1^{R3}	28					25	36

(Fig. 4). In each case, the penetrance and severity correlated with *snr1* dosage (+/− > +/+) and temperature of incubation. Similar results were obtained by using two independent *UAS:snr1-2* transgene lines.

Ectopic expression of SNR1-2 in developing wing tissues using *P[GaWB]69B-GAL4* resulted in the appearance of ectopic mechanosensory bristles, disruption of the L2 longitudinal vein (Fig. 3), and an ectopic bristle on the distal portion of the L3 vein (30% of wings examined). Typically, the L3 vein has three sensilla and no bristles. Vein-specific effects have been observed with expression of the dominant-negative *brm^{K804R}* and portions of *osa* (Collins et al., 1999; Elfring et al., 1998). Unlike *brm* and *osa*, *snr1* function does not appear to be generally limiting in the dorsal thorax, legs, and head regions. However, expression of SNR1-2 in the abdomen during late development using *P[e22c-GAL4]* resulted in lethality at 29°C in a *snr1* (+/−) background (Table 2) and significant cuticle disruption, including incomplete fusion of the tergites along the dorsal midline (Fig. 3E–H). Heat-shock GAL4-induced expression of SNR1-2, but not SNR1-1, also resulted in lethality (data not shown), confirming that expression of the truncated protein disrupted normal SNR1 functions.

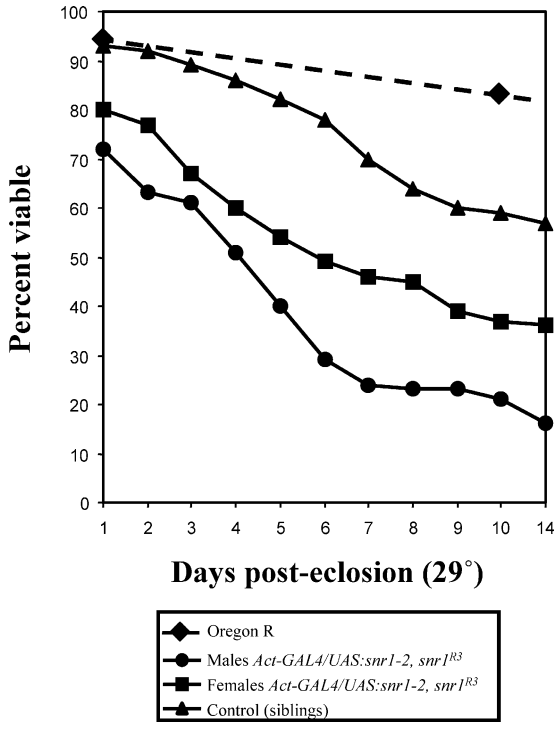

Fig. 4. SNR1 is required for sustained adult viability. Percent viability decreased significantly over time at 29°C for flies ubiquitously expressing SNR1-2. The decreased lifespan was more pronounced in males (15% viable) relative to females (35% viable) and wild type controls (88% viable) at 14 days post-eclosion.

Ubiquitous expression of SNR1-2 using *Actin5C-GAL4* showed similar patterning defects and resulted in additional phenotypes. Over 70% of females ($N = 101$) of the genotype *w; Act5C-GAL4/UAS:snr1-2, snr1^{R3}* were sterile, compared with 11% of control females ($N = 45$) under identical conditions. Surprisingly, ubiquitous expression of SNR1-2 also resulted in a significant decrease in adult lifespan (Fig. 4). Adults of the genotype *Act5C-GAL4/UAS:snr1-2, snr1^{R3}* exhibited a rapid decrease in viability following eclosion, and the effect was more pronounced among male progeny. These results were verified by using a second *Act5C-GAL4* insertion on chromosome (II), with even greater disparities in the sex ratio among progeny of the genotype *Act5C-GAL4;UAS:snr1-2, snr1^{R3}* (9:1 female; $N = 131$).

To examine whether the SNR1-2 phenotypes arose from disruption of Brm complex assembly/function or repre-

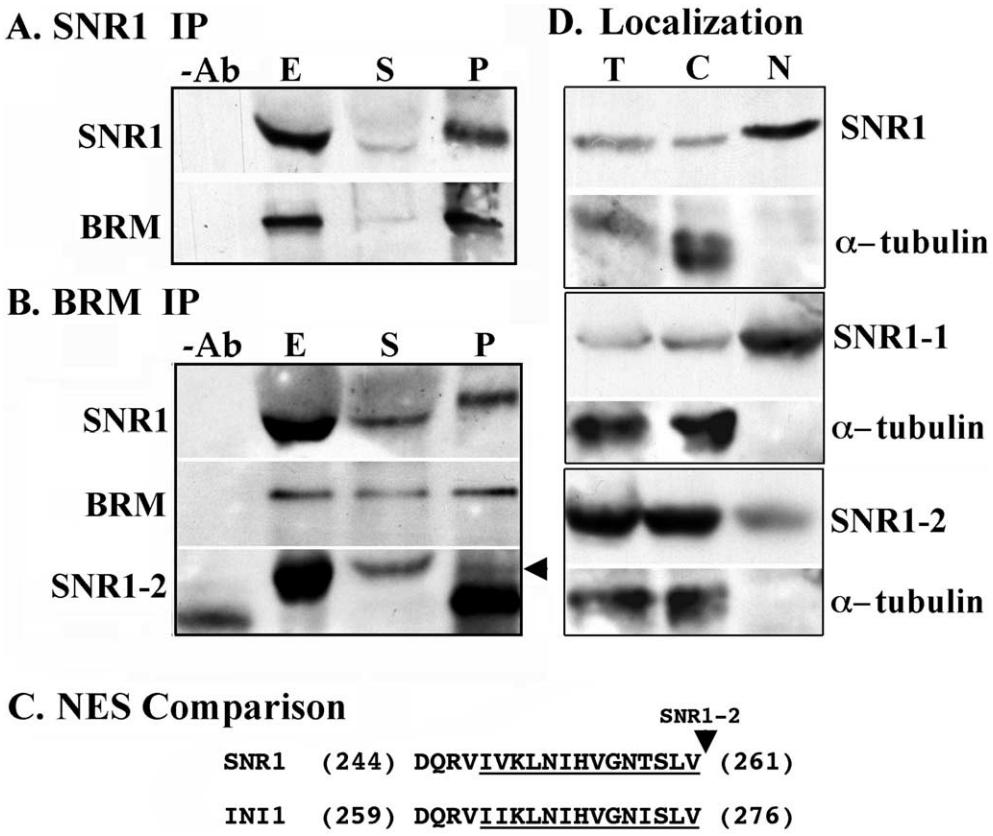

Fig. 5. SNR1-2 is incorporated into the Brm complex and is mislocalized to the cytoplasm. (A) SNR1 is stoichiometric with BRM in anti-SNR1 immune precipitates from embryonic extracts. Lanes: -Ab, G-Sepharose control with no primary antibody (lane represents 50% of pelleted material); E, 100 μg extract; S, supernatant fraction (lane represents 20% of input protein extract); P, pellet fraction (lane represents 50% of coimmunoprecipitated proteins). (B) SNR1 and a portion of SNR1-2 coimmunoprecipitate with BRM. The arrow indicates the position of SNR1-2 (~29 kDa) in the precipitated material. The stronger signal at ~25 kDa may represent a SNR1-2 degradation product; although a cross-reacting band of similar molecular mass is seen in the (−Ab) control, though not in the extract or supernatant lanes. (C) A conserved nuclear export signal (NES) is present in both SNR1 and INI1. The SNR1-2 protein terminates immediately following the NES sequence. (D) SNR1-2 mislocalizes to the cytoplasm. Equal amounts of total (T), cytoplasmic (C), and nuclear (N) extracts prepared from pupae expressing SNR1-1 and SNR1-2 or control pupae were probed with anti-SNR1 and anti-tubulin antibody.

sented dominant-negative effects specific to SNR1, we tested for incorporation of SNR1-2 into the Brm complex by coimmunoprecipitation with BRM. Consistent with earlier reports (Collins et al., 1999; Dingwall et al., 1995; Papoulas et al., 1998), the wild type SNR1 coprecipitated with BRM, and we found that BRM could efficiently coprecipitate with SNR1 (Fig. 5A). The stoichiometry of SNR1 and BRM coprecipitation was similar, using antibodies to either protein (compare Fig. 5A and B), suggesting that most SNR1 was in complexes with BRM. Extracts prepared from *P[w$^+$, UAS:snr1-2]/Act5C-GAL4 (II)* embryos were incubated with antibodies to BRM, fractionated, and probed with antibodies to SNR1. Importantly, a portion of the SNR1-2 protein was found within Brm complexes (arrow in Fig. 5B).

A nuclear export signal (NES) in INI1, when unmasked by C-terminal deletions, allows for hCRM1-dependent export in cultured cells that may be important for cellular immunity (Craig et al., 2002; Turelli et al., 2001). The NES sequence is strongly (13/14 residues) conserved in SNR1, and the SNR1-2 deletion removes residues immediately C-terminal to the NES sequence (Fig. 5C). As the majority of SNR1-2 was not found in complexes with BRM, we examined whether the protein was properly localized. Extracts prepared from pupae that ubiquitously expressed either SNR1-1 or SNR1-2 and from uninduced controls were fractionated and probed with antibodies to SNR1 and tubulin (Fig. 5D). While the majority of endogenous SNR1 and SNR1-1 protein was found in the nucleus as expected, SNR1-2 was significantly elevated in the cytoplasm. Thus, overexpression does not affect SNR1-1 localization and the NES sequence can confer nuclear export in vivo when unmasked by the deletion of C-terminal residues. Further, BRM localization was not affected following induction of SNR1-2 (data not shown), suggesting that the SNR1-2 phenotypes were the result of dominant interference with normal SNR1 function in the nucleus and/or that the SNR1-2 protein in the cytoplasm affected unknown processes. As the SNR1-2 phenotypes are sensitive to *snr1* dosage, the nuclear functions of SNR1 are most likely to be impacted; however, gain-of-function cytoplasmic functions of SNR1-2 are possible.

Developmental expression of snr1

The SNR1-2 expression phenotypes reflect disruption of late larval/early pupal development, suggesting critical Brm complex functions in metamorphosis. We therefore reexamined the *snr1* mRNA profile with emphasis on late development using highly specific riboprobes (data not shown). We previously found *snr1* mRNA present at every developmental stage, with the highest levels observed in early embryos, pupae, and virgin females (presumably in oocytes), contrasting with lower levels in late-staged embryos and larvae (Dingwall et al., 1995). Using *snr1* riboprobes, we observed a transient increase in mRNA in late third instar larvae and again in pupae, coincident with rising titers of the molting hormone 20-hydroxyecdysone that regulates metamorphosis. Unexpectedly, we also observed detectable *snr1* mRNA in adult males (data not shown). Thus, there is significant expression of *snr1* mRNA in adults and during developmental periods that show susceptibility to expression of SNR1-2.

To better define the developmental distribution of SNR1 in specific tissues, affinity-purified antibodies were used to detect the protein in third instar larval imaginal discs, salivary glands, and adult ovaries. As a control to determine whether the SNR1 polyclonal antiserum directed against the full-length protein (see Materials and Methods) was capable of detecting the protein in all tissues, SNR1 was produced ectopically in third instar larvae by using a *hsp70-snr1* cDNA transgene. Following a 60-min heat-shock with a 30-min recovery, imaginal discs were dissected, fixed, and immunostained. In heat-shocked, but not control larvae, high level expression of SNR1 was detected in all imaginal tissues, including the leg and wing discs (Fig. 6A), confirming the specificity of the antibody.

SNR1 and BRM are ubiquitously expressed in early embryos, with late embryonic expression generally restricted to the CNS and brain (Dingwall et al., 1995; Elfring et al., 1998). In striking contrast, SNR1 was not detected in most cells of the larval CNS and brain, with only a few cells in the optic lobe expressing the protein (Fig. 6B). SNR1 was only present at detectable levels in a subset of cells of the leg and wing discs, if at all (Fig. 6C and E vs 6D and F). Consistent with a genetic role for *snr1* in eye disc development (Brumby et al., 2002), SNR1 was present at high levels in the eye disc both anterior and posterior to the morphogenetic furrow (MF), as well as the developing photoreceptor cells (Fig. 6G and G′). SNR1 was also present in salivary gland nuclei, where it was associated with the polytene chromosomes (Fig. 6H–I); although, it was found at higher levels in imaginal cells compared with polytene cells of the salivary gland and gut tissues. SNR1 expression was observed in a subset of cells within the ovariole (Fig. 6J and K), specifically in the posterior portion of the germarium (regions 2 and 3), a few cells of the somatic follicular sheath, at low levels in nuclei of the nurse cells, and the developing oocyte. Therefore, SNR1 is present in cells that exhibit somatic and germline clone null phenotypes and are sensitive to the expression of SNR1-2. The absence of detectable SNR1 in cells of the leg imaginal discs that express high levels of BRM suggests that SNR1 is an essential component of a subset of Brm complexes.

BRM and OSA associate with polytene chromosomes at many sites (Collins et al., 1999), including less condensed interband regions that generally correspond to actively transcribed genes, suggesting that the Brm complex might have a primary role in opening chromatin to allow for transcript initiation at many gene loci. To address this question directly, we examined the localization of SNR1, BRM, and RNA PolII on the salivary gland polytene chromosomes (Fig. 7). Both SNR1 and BRM were broadly distributed at many sites in essentially identical patterns and at significantly higher levels in the interband regions, nearly coincident (~90%) with RNA PolII (Fig. 7D and H). In addition, neither the Brm complex nor PolII was found significantly associated with the BX-C homeotic cluster, a known in vivo target of the Brm complex that is inactive in this tissue. The largely overlapping localization patterns suggest that sites of Brm complex accumulation coincide with actively transcribed loci, although we are unable to determine the precise step at which the Brm complex interfaces with the transcription machinery as the PolII antibody recognizes both elongating and promoter bound forms (Skantar and Greenleaf, 1995). Importantly, the SNR1 and BRM proteins did not entirely colocalize with PolII. In particular, the relative intensity of PolII staining at specific sites (indicated by colored arrows in the figure) differed from either BRM or SNR1. Conversely, BRM and SNR1 also accumulated at locations not associated with PolII. Our data indicate that the Brm complex is widely present at actively transcribed loci, perhaps to facilitate aspects of gene activation or maintenance. However, while transient interactions could not be ruled out, the Brm complex is not a constitutive component of all PolII complexes in vivo and raises the possibility that the Brm complex has opposite functions to PolII at specific target genes.

snr1 functions as both an activator and repressor

A heterozygous $snr1^{R3}$ mutant shows no obvious genetic interaction with the *Pc* gene, although it moderately enhances a *trx* mutant phenotype and shows weak interaction with some *brm* and *osa* alleles (Collins et al., 1999; Dingwall et al., 1995). In addition, $snr1^{R3}$ can suppress (~50%) the antennal-to-leg transformation of the dominant $Antp^{Ns}$ mutation (data not shown), similar to the suppression observed with *brm*, *mor*, and *osa* (Brizuela and Kennison, 1997; Tamkun et al., 1992; Vazquez et al., 1999). Several trx-G genes (including *brm*, *mor*, and *osa*) can influence the expression of a variegating *white* minigene under the influence of the *Scr*

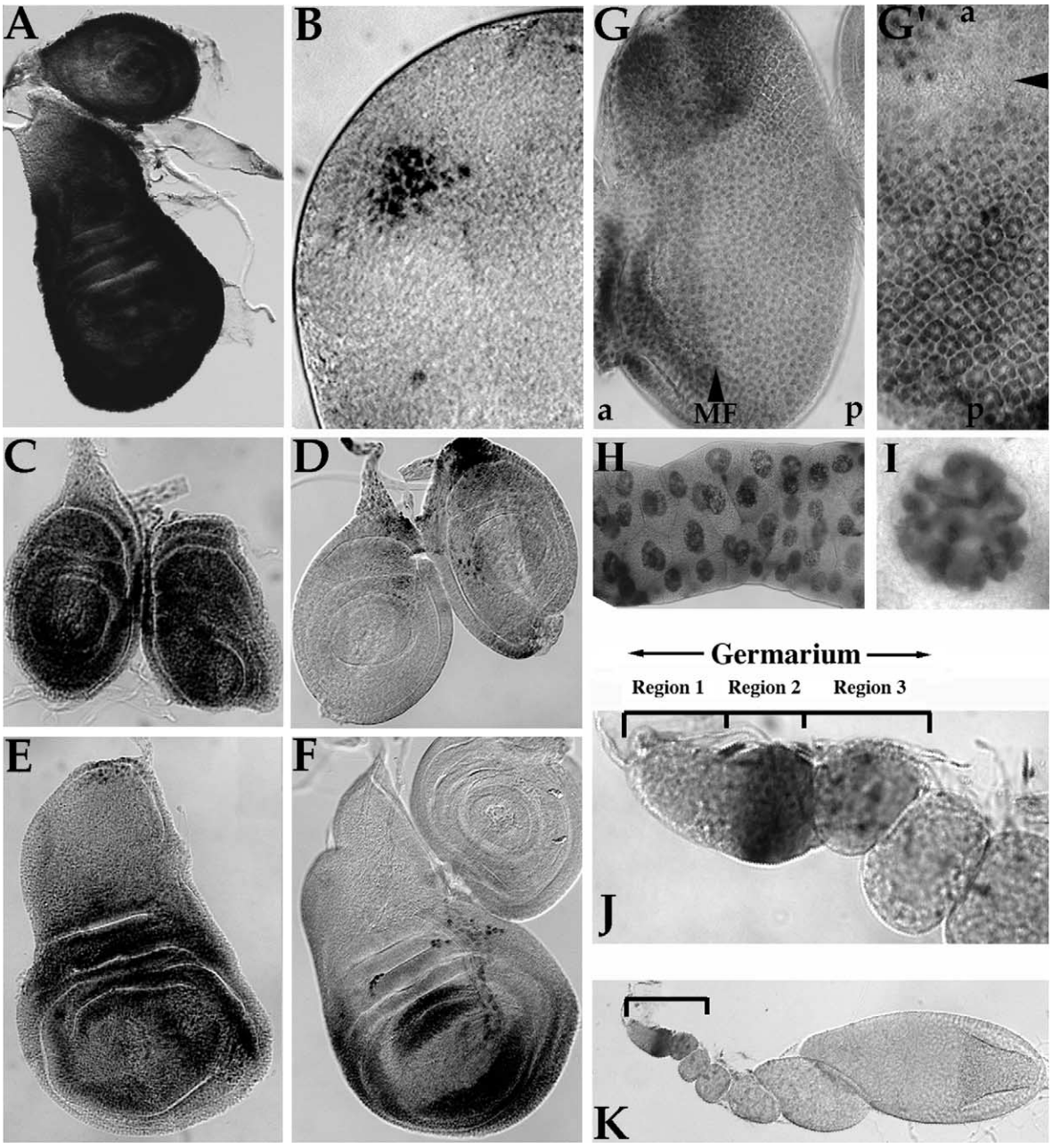

Fig. 6. SNR1 is restricted to a subset of BRM-expressing tissues. (A) Third instar larval wing and leg discs immunostained with anti-SNR1 following a 60-min heat-shock of an *hsp70-snr1* cDNA. High levels of SNR1 were detected in all larval discs. (B) SNR1 expression in the larval brain is restricted to the optic lobe. Immunolocalization of SNR1 and BRM in similarly staged third instar larval leg (C, D) and wing (E, F) imaginal discs. BRM (C, E) is expressed in all cells of the leg and wing discs, with elevated levels in the developing wing pouch. SNR1 (D, F) is not present at high levels in the T1/T2 leg discs (D), T3 leg disc (F, upper right), or the wing notum. (G, G′) Expression of SNR1 in the eye disc. The morphogenetic furrow (MF) and anterior/posterior (a/p) orientations are indicated in (G, G′). A magnified view of an eye disc region (G′) showing cells both anterior and posterior to the morphogenetic furrow (arrowhead) reveal high level expression of SNR1 in all cells except those arrested in G_1 phase within the furrow. (H, I) SNR1 is present in salivary gland nuclei. Shown in (I) is a magnified view of a single salivary gland nucleus (100× magnification). Note the punctate staining associated with the chromosomal DNA. (J, K) SNR1 is expressed at high levels in regions 2 and 3 of the germarium, though SNR1 is found at significantly lower levels in the somatic follicular sheath and developing oocyte.

(HOM gene) enhancers carried on the same transgene construct (Gindhart and Kaufman, 1995). Using the same assay with a $snr1^{R3}$ null allele, we found that *snr1* was necessary to maintain active transcription of the *white* minigene (data not shown). Thus, *snr1* can affect HOM gene expression as a coactivator of transcription.

The wide distribution of SNR1 and BRM on polytene chromosomes raised the possibility that the Brm complex

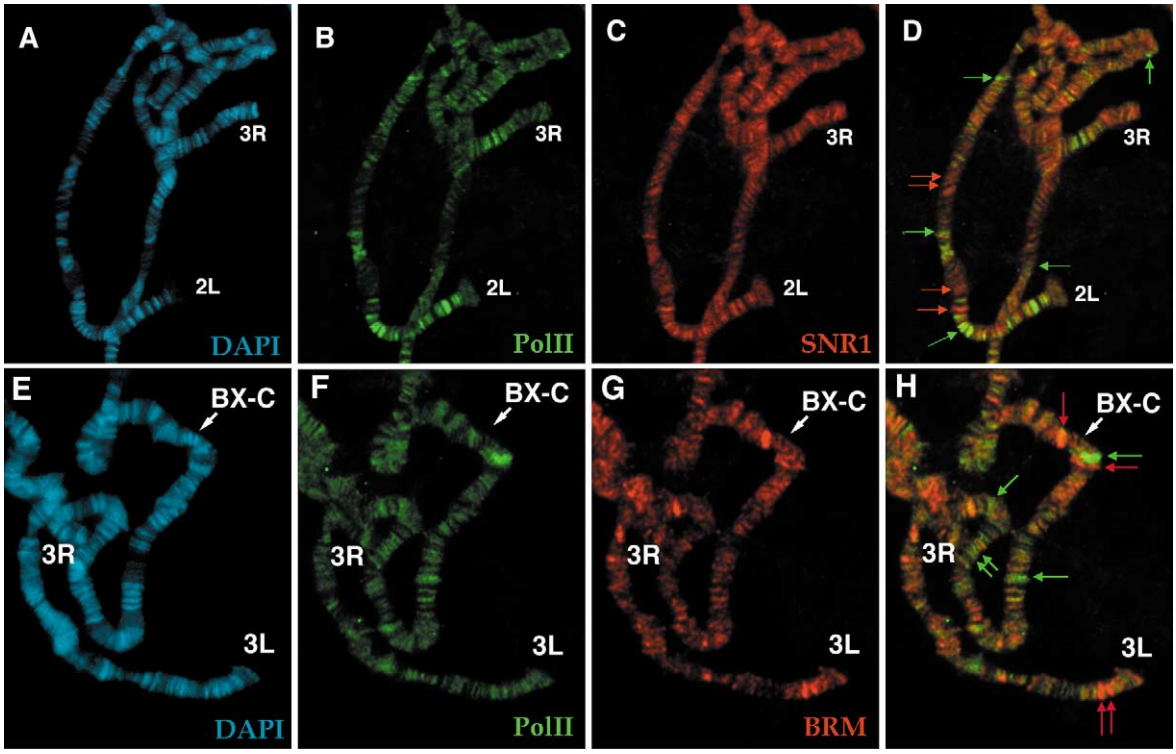

Fig. 7. SNR1 and BRM frequently colocalize with PolII on salivary gland polytene chromosomes. Immunolocalization of PolII (B, F), SNR1 (C), and BRM (G) reveals broad distribution at many sites predominantly within interbands. Colocalization of SNR1 with PolII (D) and BRM with PolII (H) at ~90% of sites. Green arrows indicate sites of strong PolII and weak or absent SNR1/BRM staining, while red arrows indicate sites of strong SNR1/BRM staining and weak or absent PolII staining. Shown is the position of the BX-C, a known Brm complex target. No strong PolII, SNR1, or BRM signals are detected at this locus, which is inactive in salivary gland nuclei. DAPI-stained preparations of the same chromosomes are shown in (A, E).

might globally affect gene expression. We examined the influence of Brm complex genes on the expression of a variegating allele of the *white* gene, w^{m4h} (Dorn et al., 1993; Reuter and Wolff, 1981). This test of position effect variegation (PEV) measures the quantitative effects of chromosomal position and higher order structure on gene expression (Wakimoto, 1998; Wallrath and Elgin, 1995). An *E(var)* mutation enhanced the variegation of w^{m4h} (Fig. 8A and B), whereas *snr1* suppressed the variegating phenotype of w^{m4h} (Fig. 8C). A $snr1^{R3}$/*E(var)3–4* combination resulted in intermediate variegation (Fig. 8D). Quantitative pigment assays were performed by using (>20) male flies heterozygous for *Su(var)205*, *E(var)3–4*, and various Brm complex gene mutations that also carried the w^{m4h} allele. Flies heterozygous for *snr1* mutations, including $snr1^{R3}$, $snr1^{SR21}$, $snr1^{SR71}$, $snr1^{E2}$, and $snr1^{E3}$ as well as a brm^{2}, $snr1^{R3}$ recombinant exhibited a strong suppression of the variegating phenotype (Fig. 8, and data not shown); however, mutations in Brm complex genes alone had little effect, or possibly a slight enhancement. As a control for genetic background effects on variegation, a viable precise excision of the *P*-transposon (Dingwall et al., 1995) that was used to derive the *snr1* mutant alleles was tested in parallel, with no difference in pigment levels observed between the $snr1^{PIrev}$ and the w^{m4h} control males (data not shown). Thus, *snr1* is epistatic to a known enhancer of variegation in this assay, suggesting that SNR1 may directly regulate certain functions of the Brm complex.

Discussion

Our genetic analyses of new *snr1* mutant alleles, ectopic expression of deletion transgenes, and developmental studies demonstrate that SNR1 is critically required for oogenesis, proper wing, abdomen, and nervous system development, and for sustained adult viability. SNR1 is highly conserved with counterparts in yeast (SNF5) and mammals (INI1) and is a component of the *Drosophila* Brm (SWI/SNF) complex purified from embryos. It serves as an essential subunit for some Brm complex activities; however, SNR1 appears to be an optional component. In particular, we found that *snr1* was not genetically required for proper leg morphogenesis and that SNR1 was not expressed at detectable levels in the late larval leg discs, despite strong evidence for essential Brm complex functions in regulating leg development (Collins and Treisman, 2000; Kennison and Tamkun, 1988; Tamkun et al., 1992).

While the biological functions of SNR1 and INI1 are complex, both are essential for development. Specifically, genetic analyses of both *snr1* null and conditional mutants and disrupting SNR1 function with dominant negative

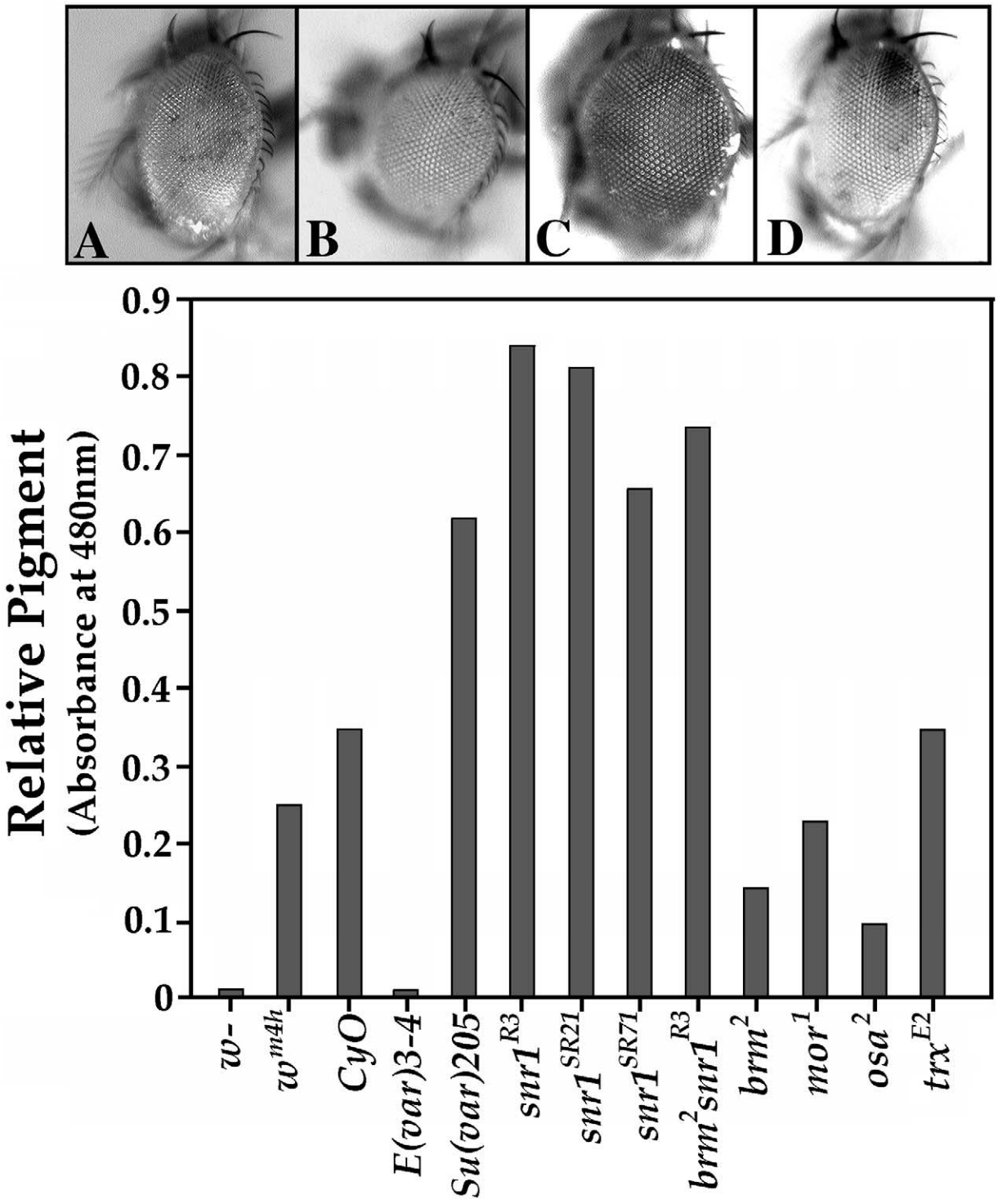

Fig. 8. *snr1* functions as a suppressor of w^{m4h} variegation. Relative eye pigmentation observed in flies of the following genotypes: w^{m4h};+/+ (A), w^{m4h};*E(var)3–4*/+ (B), w^{m4h};$snr1^{R3}$/+ (C), w^{m4h};*E(var)3–4*/$snr1^{R3}$ (D). (Bottom) Quantitative pigment assays (absorbance units at 480 nm) from male w^{m4h} flies carrying heterozygous mutant alleles of the genes shown.

transgenes have shown that SNR1 is required at all developmental stages and in adults, though in a restricted set of tissues. Disruption of INI1 function blocks early murine embryogenesis at the peri-implantation stage (E3.5), and chimeric mice harboring an *ES* cell-derived *INI1* knockout develop tumors of the central nervous system and soft tissue sarcomas at high frequency (>30%) (Klochendler-Yeivin et al., 2000), suggesting cell lineage specificity. Moreover, mutations in INI1 are strongly (~90%) associated with aggressive childhood cancers (Sevenet et al., 1999; Ver-

steege et al., 1998), suggesting a critical role in restricting cell growth, a role we have demonstrated for *snr1* as well (Marenda et al., 2003).

Developmental requirements for snr1 function

A single *snr1* transcript is present at all developmental stages and in adults (Dingwall et al., 1995). SNR1 protein and RNA levels fluctuate during development, with elevated expression in specific tissues. Our genetic studies of *snr1* revealed that null alleles caused early larval lethality. However, both *snr1* germline clones and maternal expression of SNR1-2 caused female sterility and late embryonic lethality, implying both a critical role in early development and that a large maternal contribution of *snr1* mRNA was sufficient for embryogenesis. An unexpected result of our studies was the finding of an important role for SNR1 in sustained adult viability, including males. Consistent with this view, we found *snr1* mRNA present in male flies (our unpublished results); moreover, both *mor* mRNA (Crosby et al., 1999) and BRM protein (Elfring et al., 1998) have been found in adult males, raising the possibility that the Brm complex may be continuously required.

The *snr1* somatic and germline clone phenotypes largely overlap with those observed for *brm* (Elfring et al., 1998), and effects caused by expression of interfering transgenes suggest that *snr1*, *brm*, and *osa* are important for proper wing development. Phenotypic differences likely reflect unique tissue-specific functions for *snr1*. For example, analysis of somatic clones revealed that *snr1*, like *brm*, was required for proper peripheral nervous system development, as both *snr1* and *brm* mutant clones exhibited twinned, missing, stunted, and fused bristles in the abdomen reminiscent of abnormalities associated with mutations in neurogenic genes, such as *Notch* (Hartenstein and Posakony, 1990).

Both somatic clonal analyses and ectopic expression of SNR1-2 revealed that histoblast proliferation was strongly compromised when functional *snr1* was limiting, and the *snr1* somatic clone phenotype was enhanced by the presence of a *brm* mutation, suggesting important functions for the Brm complex in histoblasts. Clusters of histoblasts within each segment produce the integument of the adult abdomen on both the dorsal and ventral surfaces. The histoblasts proliferate during pupal development, with division initiated shortly after pupariation (AP) and the commencement of migration at about 14 h AP (Fristrom and Fristrom, 1993). Fusion of the different anterior and posterior dorsal histoblast nests that produce the tergite occurs between 18 and 40 h AP. The fusion of the dorsal cuticle was most severely affected by expression of SNR1-2 that correlated with both the temperature of incubation and the *snr1* dosage, suggesting that SNR1 serves to guide Brm complex functions in the developing histoblasts. Although the genes regulated by *snr1* are unknown, one possibility is the *escargot* gene that encodes a zinc-finger protein required for maintaining a diploid state in imaginal cells and normal development of the abdomen (Fuse et al., 1994; Hayashi, 1996; Hayashi et al., 1993). High-level expression alone could not account for the SNR1-2 phenotypes, as SNR1-1 and rescue transgenes had no effect. While formally possible, it appears unlikely that SNR1 has normal functions independent of the Brm complex.

In addition to the bristle defects observed in *snr1* clones, expression of SNR1-2 disrupts normal wing vein patterning and PNS development. The observed defects all coincide with a developmental period in late larvae and early pupae associated with rapid cell proliferation and differentiation and closely correlate with increased expression of Brm complex genes. Of possible significance, *snr1* mRNA expression fluctuates coincident with transient pulses of ecdysone in late development (our unpublished results), especially as the SWI/SNF complex can assist the activation potential of other steroid hormone-binding transcription factors (Peterson and Workman, 2000). Similar to expression of BRM^{K804R} that disrupts ATPase activity in vivo but not the formation or stability of the complex, the expression of SNR1-2 resulted in specific wing vein patterning defects, including disruptions along the L2 vein and ectopic bristles on the L3 vein. Although some of the misexpression phenotypes overlap, the SNR1-2 effects were most striking in anterior wing veins (L2 and L3), while BRM^{K804R} phenotypes affected the entire wing, including the L5 vein and posterior crossvein, as well as the wing margin. The expression patterns of SNR1 and BRM are nearly identical in the larval and pupal wing disc (our unpublished observations); thus, SNR1 appears to be important for Brm complex activities in restricted regions of the developing wing.

An unexpected finding was an important role for SNR1 in sustained adult viability. Similar to effects caused by expression of SNR1-2, flies rescued with a heat-shock cDNA had a dramatically reduced viability following eclosion (<3 days). The reduced longevity is unlikely due to nonspecific effects, such as accumulation of overexpressed proteins, as our temperature-sensitive *snr1* mutant also exhibited shortened lifespans (<7 days), and this effect was rescued by additional copies of the wild-type gene (Marenda et al., 2003). Interestingly, *ash1*, another member of the trx-G that genetically interacts with both *brm* and *trx*, is also required for adult viability (Landis et al., 2001). ASH1 is a component of a large ($\sim$2 MDa) complex distinct from the Brm complex (Papoulas et al., 1998), but whose composition is unknown. As *ash1* is important for *trx* function (Rozovskaia et al., 1999) and SNR1 physically and genetically interacts with TRX (Marenda et al., 2003), it may be possible that ASH1 can also form transient complexes with SNR1 in vivo. Thus, shortened adult viability may reflect reduced gene transcription due to disruption of Brm complex activity or another complex, such as Ash1.

SNR1 is a component of a subset of brm complexes

The SNR1/INI1 subunit copurifies with the fly and mammalian SWI/SNF complexes (Papoulas et al., 1998; Wang et al., 1996), and the presence of INI1/hSNF5 helps to reconstitute full in vitro chromatin remodeling activity (Phelan et al., 1999), suggesting that SNR1/INI1 is a core component. BLAST database searches and reduced stringency hybridization confirmed that *snr1* is the only *SNF5*-related gene in flies (our unpublished results, and Papoulas et al., 1998). Unlike loss-of-function mutations in *brm, mor,* and *osa* that encode other subunits of the Brm complex, *snr1* null alleles are not significantly dosage-limiting in some sensitized genetic assays. For example, *snr1* null alleles do not suppress a *Pc* mutant phenotype in the male prothoracic leg, an assay that is often used to define members of the trx-G of activators (Kennison, 1995).

Consistent with our genetic studies of *snr1,* most Brm complex components are also not encoded by previously identified trx-G genes (Papoulas et al., 1998). Some of the individual subunits may exist in excess of that required for the formation or function of the complex, they may be required for specific functions of the Brm complex, or they may be components of other complexes, making null phenotypes difficult to interpret. Further, depending on the specific target gene or tissue, a subunit may have different regulatory functions on complex activities (activation or repression); thus, removal of the subunit may have unanticipated effects. SNR1 and BRM efficiently coprecipitate; however, SNR1 is not detectably expressed in all tissues where BRM is found at high levels (Elfring et al., 1998). Unlike BRM, SNR1 is not found at elevated levels in the notum portion of the third instar larval wing disc that gives rise to dorsal thorax, and *snr1* mutant clones revealed only minor thoracic bristle defects that may represent loss of *snr1* function in a restricted set of developing PNS cells. Similarly, our *snr1* conditional mutant did not display any significant thoracic defects at any temperature (Marenda et al., 2003). While we cannot rule out that earlier expression of *snr1* RNA or protein allows for the completion of normal thoracic development after clone induction, ubiquitous expression of SNR1-2 also did not elicit thoracic phenotypes.

Strikingly, SNR1 was not present at detectable levels in the leg discs, there were no SNR1-2 phenotypes in the legs, nor has there been any evidence for a genetic role of *snr1* in the development of the legs (Dingwall et al., 1995; Marenda et al., 2003), despite convincing requirements for other Brm complex genes (Brizuela and Kennison, 1997; Collins et al., 1999; Elfring et al., 1998; Kennison and Tamkun, 1988; Tamkun et al., 1992). In addition to an absence of detectable *snr1* clone or dominant-negative expression phenotypes in legs, genetic interaction tests using a *snr1* null allele have failed to reveal any function for *snr1* in suppressing the ectopic sex comb phenotype associated with mutations in several Pc-G genes, including *Pc* (Pc^1, Pc^3, Pc^4), Pcl^{11} and $E(z)^{60}$ (our unpublished observations). Furthermore, an unusual allele of the Pc-G gene *E(z)* that mimics *trithorax* mutant phenotypes [$E(z)^{Trm}$], including similar genetic interactions, showed enhanced mutant phenotypes in the abdomen and legs in combination with alleles of *brm, mor,* and *osa*; however, only the abdomen phenotype was enhanced by *snr1* (Bajusz et al., 2001). Thus, SNR1 is not required for Brm complex functions in leg development and, perhaps similar to what has been proposed for OSA (Collins et al., 1999), it appears to be an optional component in a subset of Brm complexes.

Our finding that SNR1-2 expression phenotypes were sensitive to *snr1* dosage and that the truncated protein was found predominantly in the cytoplasm raised the possibility that SNR1-2 interfered with normal SNR1 function by antagonizing specific interactions between the Brm complex and cellular proteins. It appears unlikely that SNR1-2 phenotypes result from mislocalization of the Brm complex, as we did not observe any cytoplasmic redistribution of BRM following induction of SNR1-2 (our unpublished results). Expression of large SNR1 deletions had no phenotype, suggesting that expression of the N-terminal residues alone was not deleterious, a view supported by our analyses of *snr1* mutant alleles that had similar truncations. The Repeat regions in SNR1 and INI1 have been shown to mediate specific protein contacts, acting independently or in concert to promote the recruitment of the metazoan SWI/SNF complexes to target loci (Cheng et al., 1999; Morozov et al., 1998; Rozenblatt Rosen et al., 1998; Takayama et al., 2000). For example, HIV integrase, the Epstein–Barr viral protein EBNA2, c-MYC, and the human papillomavirus (HPV) E1 protein required for replication of the viral genome all interact with INI1 through the repeat regions (Cheng et al., 1999; Kalpana et al., 1994; Lee et al., 1999; Morozov et al., 1998; Wu et al., 1996).

Interactions have been identified between SNR1/INI1 and the SET domains of the *Drosophila* trithorax (TRX) and human trithorax (HRX) proteins (Rozenblatt Rosen et al., 1998) that function as transcriptional regulators of the homeotic genes. TRX physically interacts with SNR1 through conserved residues within the Repeat 2 region, and their interaction is important for proper development (Marenda et al., 2003; Dingwall et al., 1995). As SNR1-2 can assemble into Brm complexes and TRX did not associate with SNR1-2 in vivo (data not shown), the observed phenotypes might reflect diminished functions of TRX or other factors. The activities of other cellular proteins are also likely to require SNR1, including transcription factors such as Bicoid (our unpublished data) and the cell cycle regulators Cyclin E/CDK2 (Brumby et al., 2002), and these may be candidates for functional disruption by SNR1-2. One possibility for a protein shown to associate directly with Repeat 1 is c-MYC, whose transactivation activity depends on interaction with INI1 (Cheng et al., 1999; Takayama et al., 2000). Although a functional homolog of c-MYC exists in flies (Gallant et al., 1996; Johnston et al., 1999), it is unknown whether a similar relationship exists between the *Drosophila* counter-

parts or whether *Drosophila* c-MYC activity is affected by expression of SNR1-2. Another possibility is that SNR1-2 protein in the cytoplasm associates with unknown proteins and blocks their activity or transport into the nucleus, reflecting novel functions of the SNR1-2 protein. Further tests are necessary to resolve among these and other possibilities.

Mounting evidence suggests that Brm complex subunits have specific roles in defining the range of targets and developmental functions of the complex. Mutations in *mor* result in midgut abnormalities and reduced eye size (Brizuela and Kennison, 1997). Ectopic expression of OSA affects wing vein patterning and disrupts eye development, and mutations in *brm*, *mor*, and *snr1* can modulate those phenotypes (Collins et al., 1999). Genetically, *osa* functions in concert with *brm* in wing tissues, but with opposite effect in eyes, and *osa* has been shown to have genetic functions distinct from both *brm* and *mor* in oogenesis (Vazquez et al., 1999). Similarly, some *snr1* mutant phenotypes overlap with those of *brm*, and *brm* can suppress many of the *snr1* conditional mutant phenotypes (Marenda et al., 2003).

Our data demonstrate a striking correspondence between localization of SNR1/BRM and RNA PolII, suggesting a critical role for the Brm complex in mediating global aspects of gene transcription regulation. Importantly, the Brm complex accumulates at sites that are not being actively transcribed, and it is not always associated with active PolII transcription. While the Brm complex likely assists PolII activity at many gene loci, it is not a core constituent of RNA PolII holoenzyme, as has been suggested (Wilson et al., 1996); thus, the Brm complex may have other roles in regulating gene expression. Consistent with reports that the yeast SWI/SNF complex can function in both gene activation and repression (Sudarsanam et al., 2000), the ectopic wing veins observed in flies overexpressing SNR1-2 are reminiscent of effects caused by derepression of specific wing vein regulators (Bier, 2000). Further, loss of *INI1/hSNF5* results in derepression of *cyclinD1* expression in rhabdosarcoma tumor cell lines, possibly due to diminished recruitment of a deacetylase activity by INI1 to the *cyclin D1* promoter (Zhang et al., 2002).

Position effect variegation (PEV) is the mosaic expression of a gene that has been moved to another position on the chromosome and is a model system used extensively to examine the effects of higher order chromosomal structure on gene expression (Grigliatti, 1991; Reuter and Spierer, 1992; Weiler and Wakimoto, 1995). Although many genes can influence the variegating phenotypes associated with classical PEV, we found that *snr1* exerts an effect as strong as known suppressors of PEV *[Su(var)205]*, and it genetically interacts with (suppresses) a known enhancer of PEV *[E(var)3-4]*. Mutations in *snr1* suppress the variegating eye pigmentation phenotype associated with $In(1)w^{m4h}$, while mutations in *brm*, *mor*, and *osa* exhibit a slight enhancing effect. Among the explanations for this difference, likely possibilities are that SNR1 may be responsible to target repressor functions of the Brm complex to specific gene loci or that it functions to directly restrict or limit complex activities.

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